Relationships between Structure and Kinetics of Cyclization of 2-Aminoaryl Amides: Potential Prodrugs of Cyclization-Activated Aromatic Mustards

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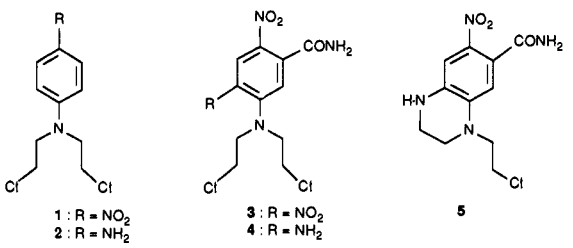
2-Nitroaryl amides of general structure **I** are proposed as bioreducible prodrugs, capable of releasing cytotoxic aminoaniline mustards **V** on bioactivation by spontaneous cyclization of the resulting 2-aminoaryl amides **II** via a tetrahedral intermediate, **III**. This concept allows separate optimization of the substituent effects influencing nitro-group reduction and mustard reactivity. A series of model 2-aminoaryl amides has been synthesized, and their rates of cyclization have been studied; these varied by a factor of more than 50 000-fold (k_{obs} from 0.00040 to 21 min^{-1}) at pH 2.4. For three compounds studied in detail, the rates were linearly dependent of pH, indicating that no change in the mechanism of the rate-determining step occurs over the pH range studied. The nucleophilicity of the amino group had a modest influence on the kinetics of cyclization, with electron-withdrawing groups slowing the rate. The geometry of the compound was also important, with structure–activity relationships indicating that the rate of cyclization is greatly enhanced by the preorganization of the molecule. In contrast, 4-substitution on the leaving aniline by a variety of groups had little effect on the cyclization reaction. These results are consistent with the rate-determining step being formation of the tetrahedral intermediate. These model studies suggest that the phenyldimethylacetamide system could be developed as a prodrug system for the bioreductively-triggered release of amines. Further substantial rate enhancements appear possible by alterations in the geometry of the system, whereas substitution of electron-withdrawing groups (required to raise the nitro-group reduction potential into the appropriate range) has only relatively modest retardation effects on rates of cyclization. More rigid systems may also be useful; a nitronaphthaleneacetamide analogue cyclized spontaneously during nitro-group reduction, suggesting a very short half-life for the reduced intermediate (amine or hydroxylamine).

Compounds which can be selectively activated in the hypoxic regions of solid tumors to generate potent cytotoxins capable of diffusing to kill surrounding oxygenated tumor cells are of interest as potential tumor-selective drugs.[1] One design for such hypoxia-selective cytotoxins (HSCs) is the class of nitroaromatic mustards,[2] which are activated by reduction of the nitro group through electron release to the mustard. We have shown that considerable control can be exercised over the design of such compounds, and examples have been reported which possess considerable selectivity for hypoxic compared with well-oxygenated mammalian cells in culture.[3] However, a limitation of this design is that both the nitro group and the alkylating unit are attached to a common aromatic system but have opposing electronic requirements. To achieve sufficiently rapid enzymic reduction of aromatic nitro groups requires reduction potentials above ca. −450 mV,[2] and in the benzene system, this requires a very electron-deficient ring.

For example, the 4-nitroaniline mustard **1** shows only minimal hypoxic selectivity, even though the putative active form (the amine **2**) is very cytotoxic (IC_{50} 0.07 μM); this has been attributed to very slow cellular reduction of **1**, which has a low reduction potential (ca. −520 mV).[4] The dinitro carboxamide mustard **3**, possessing two additional electron-withdrawing groups, has a more appropriate reduction potential (ca. −460 mV) and shows considerable hypoxia selectivity in vitro.[3] However, this electron deficiency greatly lowers the cytotoxicity of the corresponding putative active form **4**, which has a predicted[4] IC_{50} of ca. 10 μM (although this is difficult to determine because of its ready cyclization to **5**).[5] In order to maximize both the rates of bioreduction and the reactivity of the reduced (active) species, it appears necessary to electronically decouple the nitro group and the mustard.



1: R = NO_2
2: R = NH_2
3: R = NO_2
4: R = NH_2
5

We suggest a new design (Scheme 1) for bioreductively-activated mustards which achieves this decoupling and in which the reduction potential of the nitro group and the absolute reactivity (and therefore the cytotoxicity[2,4]) of the mustard can be separately manipulated by the substituents R_1 and R_2. In this design, reduction of the nitro amide **I** to the corresponding amine **II** is followed by a spontaneous cyclization to the tetrahedral intermediate **III**. Breakdown of this results in formation of the lactam **IV** and release of the amine **V**. The net conversion **I**–**V** would expel an aromatic mustard bearing an amine substituent, which will be a much more reactive alkylating agent than the preceding amide, due to enhanced electron release. The differential cytotoxicity between simple aniline mustards bearing 4-NHCOR (Hammett σ_p value 0.0) (e.g., **I**) and 4-NH_2 (Hammett σ_p value −0.66) (e.g., **V**)

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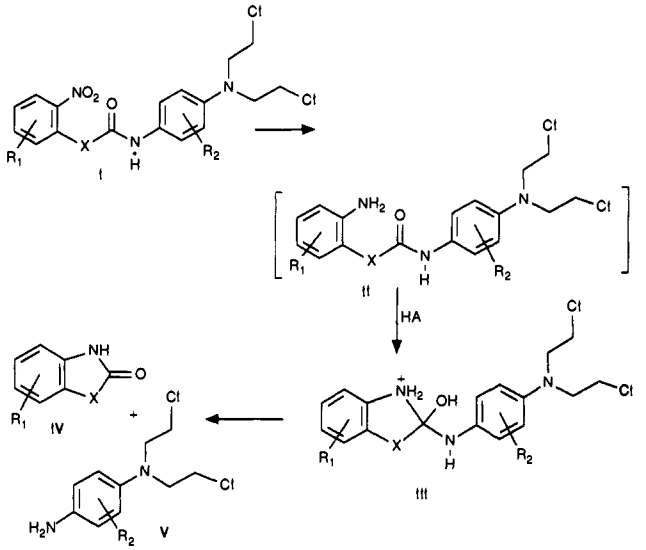
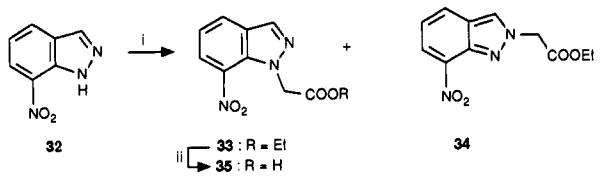
Scheme 1. Proposed Mechanism of Cyclization of 2-Aminoaryl Amides



Scheme 2[a]



[a] (i) $BrCH_2COOEt/K_2CO_3$; (ii) NaOH.

groups can be calculated by eq 1[4] as about 60-fold,

$$\log IC_{50} = 2.68\sigma + 0.15 \quad (1)$$

and this conversion (amide to amine) has been exploited previously for the enzymic release of activated mustards from prodrugs.[6]

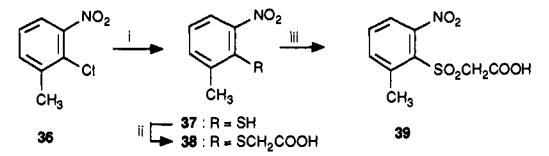
The key to the concept of Scheme 1 being successful is that the cyclization process occur at a suitable rate (a half-life of no more than a few minutes for the amine **II**) under physiological conditions. This paper reports the preparation and evaluation of the kinetics of cyclization of model 2-aminoaryl amides **7b–31b** (Table 1) in a search for structures which fulfill this requirement.

Chemistry

The 2-nitroaryl amides **7a–31a** (Figure 4) were prepared by coupling of the requisite acids and amines, using two different methods. The preferred route (method A) was by diethylcyanophosphonate-mediated coupling; use of 1-methylimidazole as the base instead of the usually employed[7] triethylamine gave superior yields in most cases. When this method was not successful, activation with $SOCl_2$/DMF gave moderate yields of the required amides.

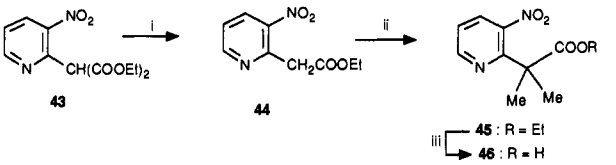
Most of the required acids are known compounds. Alkylation of 7-nitroindazole (**32**) with ethyl bromoacetate gave a mixture of the *N*-acetates **33** and **34** (Scheme 2). These were separated by chromatography, and the former was hydrolyzed with base to give 7-nitroindazole-1-acetic acid (**35**) (required for the preparation of **28a**). [(2-Methyl-6-nitrophenyl)sulfonyl]acetic acid (**39**) (for preparation of **23a**) was synthesized as shown in Scheme 3. 1-Methyl-2-chloro-3-nitrobenzene (**36**) was treated with Na_2S/DMSO to give the thiol **37**. Reaction of this with chloroacetic acid and oxidation of the resulting thioacetic acid **38** with peracetic acid gave the required sulfonylacetic acid **39**. 2-Methyl-2-(2-nitrophenyl)propanoic acid (**40**) (for the

Scheme 3[a]



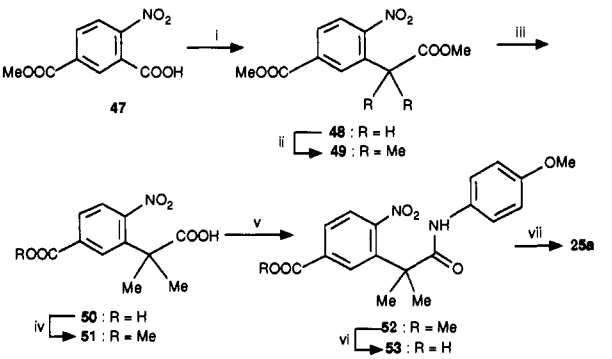
[a] (i) $Na_2S \cdot xH_2O$/DMSO; (ii) $ClCH_2COOH$/NaOH/90 °C; (iii) H_2O_2/AcOH/80 °C.

Scheme 4[a]



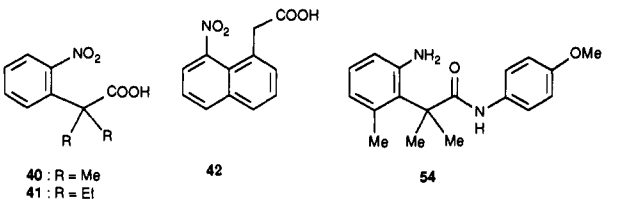
[a] (i) DMSO/H_2O, 150 °C; (ii) MeI/NaH/18-crown-6; (iii) concentrated H_2SO_4, 80 °C.

Scheme 5[a]



[a] (i) $SOCl_2$/DMF, then CH_2N_2, then Ag_2O/MeOH, heat; (ii) MeI/NaH/18-crown-6; (iii) NaOH/MeOH/H_2O, heat; (iv) MeOH/concentrated H_2SO_4; (v) $SOCl_2$/DMF, then 4-methoxyaniline; (vi) NaOH/MeOH/H_2O, heat; (vii) 1,1'-carbonyldiimidazole/DMF, then $H_2N(CH_2)_2NMe_2$.

preparation of **12a**, **13a**, and **15a–18a**) and 2-ethyl-2-(2-nitrophenyl)butanoic acid (**41**) (for the preparation of **14a**) were made by concentrated H_2SO_4 hydrolysis of the corresponding known[8] methyl esters. 8-Nitronaphthalene-



1-acetic acid (**42**) (for the preparation of **27a**) was synthesized from 1-(bromomethyl)-8-nitronaphthalene[9] via the nitrile. 2-Methyl-2-[2-(3-nitropyridyl)]propanoic acid (**46**), for preparation of **24a**, was prepared by decarbalkoxylation of diethyl [2-(3-nitropyridyl)]malonate (**43**) and dimethylation of the resulting ethyl [2-(3-nitropyridyl)]acetate (**44**) with MeI and 18-crown-6 followed by acid hydrolysis of the resulting ester **45** (Scheme 4). To prepare **25a**, we prepared methyl 5-(methoxycarbonyl)-2-nitrophenylacetate (**48**) from 5-(methoxycarbonyl)-2-nitrobenzoic acid (**47**) under Arndt–Eistert conditions and this was then methylated as above to give the α,α-dimethyl analogue **49** (Scheme 5). Base hydrolysis gave the diacid **50**, and Fischer esterification of this selectively methylated the aromatic acid to give **51**. The corresponding acid chloride was coupled with 4-methoxyaniline, the resulting amide **52** was hydrolyzed to the acid **53**, and this was coupled with *N*,*N*-dimethylethyl-

Table 1. Kinetic Data for Cyclization of the 2-Amino Amides **7b–31b** Corresponding to the 2-Nitro Amides **7a–31a** Whose Structures Are Listed in Figure 4

	pH 6.8		pH 2.4	
no.	k_{obs} (min^{-1})[a]	$t^{1/2}$ (min)[b]	k_{obs} (min^{-1})	$t^{1/2}$ (min)
7b	c		0.0026	260
8b	c		0.0080	87
9b	c		0.012	58
10b	c		0.022	32
11b	c		0.043	16
12b	0.0096	72	10.0	0.07
13b	*d*		*d*	
14b	0.028	250	1.64	0.43
15b	0.020	35	21.0	0.033
16b	0.0097	71	*e*	
17b	*f*		*f*	
18b	0.020	35	21.0	0.033
19b	*c*		*c*	
20b	0.0009	800	2.5	0.28
21b	c		c	
22b	c		c	
23b	c		0.00040	1700
24b	0.0013	530	0.078	8.9
25b	0.00090	770	2.58	0.27
26b	c		0.11	6.3
27b	*d*		*d*	
28b	c		0.0084	83
29b	c		0.0094	74
30b	*c*		0.019	36
31b	*d*		*d*	

[a] k_{obs} = observed pseudo-first-order rate constants for cyclization reaction (see text). [b] $t^{1/2}$ = half-life for cyclization reaction (= 0.693/k_{obs}). [c] No cyclization detected over 4 h. [d] Significant cyclization occurred during nitro reduction, precluding isolation of the pure amine and determination of rate constants. [e] Cyclization of the pure amine too rapid to be determined at pH 2.4 ($t^{1/2}$ < ca. 2 s). [f] Very slow nitro reduction, precluding isolation of the pure amine.

enediamine to give the required compound **25a**. A more direct route to **25a**, via initial alkylamide formation on the isomeric methyl ester of **51**, was not successful.

Kinetic Measurements. The observed pseudo-first-order rate constants of cyclization of the corresponding amines **7b–31b** of the 2-nitroaryl amides **7a–31a** at pH values of 6.8 and 2.4 are given in Table 1. The higher pH value was chosen to approximate the extracellular pH found in the vicinity of solid tumors in vivo, the physiological conditions in which such prodrugs would need to function. Because many of the compounds cyclized very slowly at this pH, measurements were also made at pH 2.4 where rate data could be collected for all analogues to elucidate structure–activity relationships for these acid-catalyzed reactions. All cyclizations were carried out in aqueous buffer of ionic strength I = 0.01 M at 37 °C and were concurrently monitored by UV/vis spectrophotometry and high-performance liquid chromatography. Rate constants are the average of those derived from UV/vis and HPLC data, with the result being discarded if the two measurements did not agree within ~5%. Where reactions proceeded too rapidly to allow enough data points for HPLC determination of the rate constant, only UV/vis data were used, with HPLC identification of products to confirm that cyclization was occurring. Reactions were monitored for 4 h in the first instance, and if cyclization products were observed, the reaction time was extended to ~4 half-lives to evaluate the rate constant. pH profiles were determined under conditions of constant ionic strength (I = 0.10 M, adjusted by addition of NaCl) and constant total buffer species concentration (0.02 M) at 37 °C. Measurements were carried out in triplicate and averaged.

Results and Discussion

Reactions involving the reduction of nitro amides, and their subsequent internal cyclization, have been previously investigated either as a method for stepwise degradation of amino acids[10,11] or as an amine-protecting device.[12,13] The reaction was found to be acid-catalyzed, with the amine the likely reduced species undergoing cyclization. Entwistle[12] showed that the reduction of nitro amides such as *N*-(3-quinolyl)-3-(2-nitrophenyl)propanamide (**10a**) in refluxing EtOH was followed by facile cyclization. This suggested that (2-nitrophenyl)alkanamides of this type might be utilized as a prodrug system.

However, in the present study, the related amine *N*-(4-methoxyphenyl)-3-(2-aminophenyl)propanamide (**9b**) was found to cyclize very slowly under physiological-type conditions. Cyclization products were observed after prolonged reaction (10 days, pH 7, 37 °C, I = 0.01 M), but first-order kinetics were not followed. The lactam and 4-methoxyaniline products were formed in nonstoichiometric amounts (along with a further unidentified product), indicating that another reaction (most likely amide hydrolysis) was competing significantly on this reaction time scale. When the desamino analogue of **9b** (a compound unable to cyclize) was monitored under identical conditions, the concentration of this starting material fell to 15% of the original concentration within 8 days.

However, although the pseudo-first-order rate constant of cyclization of **9b** could not be accurately determined, it was clear that substantial acceleration of the rate of cyclization of the reaction was required if this class of compounds was to be utilized as a prodrug system. The rate of cyclization of the 2-aminoaryl amides of general structure **II** can conceptually be influenced by changes in any of three areas of the molecule, and these are discussed separately below with reference to the model compounds **7b–31b**.

Effects of Substitution in the Amine-Bearing Ring. If formation of the tetrahedral intermediate (**III**) is rate-determining (or occurs prior to the rate-determining step), substitutions in the aromatic ring bearing the amino group are likely to have a substantial influence by altering the nucleophilicity (and pK_a values) of the resultant amines (electron-donating substituents should increase reaction rates and vice versa). It is expected that only the neutral amine species is kinetically active, as the protonated form has no unshared electron pair to act as a nucleophile. The pK_a values of **7b** and **11b** have been determined as 3.4 ± 0.2 and 3.24 ± 0.08, respectively. Figure 1 shows the pH dependence ($k_c = k_{obs}(1 + [H_3O^+]/K_a)$ of compounds **7b** and **11b** (respectively uncorrected and corrected for amine protonation). The cyclization of **12b** is presented uncorrected in both plots, due to difficulties in measuring the pK_a. The slight change in slope observed for all compounds at pH 5.8 corresponds to a change in the buffer species from acetate to phosphate. Other studies (unpublished) reveal that the buffer-independent rate profile shows no change in slope within the pH range studied. Data obtained at pH 2.4 will therefore be influenced by the extent of amine protonation, while data at pH 6.8 should not. Figure 1 indicates that no change in the rate-determining step occurs within the pH range studied. Therefore, it is expected that both data sets will show similar structure–activity relationships and that the broad conclusions drawn (from the larger body of data) at pH 2.4 will also apply at the physiologically-relevant pH of 6.8. The rates of cyclization decline steadily with in-

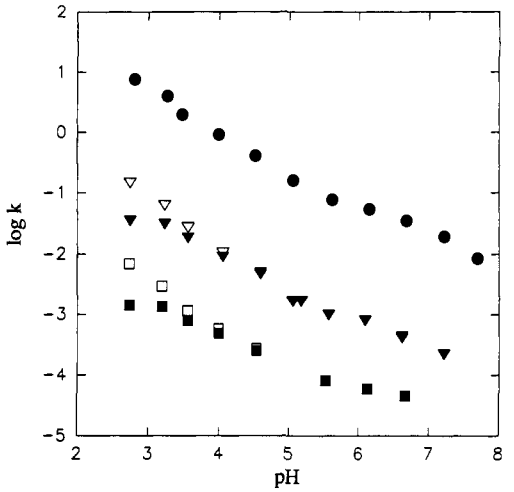


Figure 1. pH dependence of the observed pseudo-first-order rate constant of cyclization: (■) **7b**, (▼) **11b**, and (●) **12b**. Hollow symbols represent data corrected for amine protonation. Rate constants were evaluated in triplicate with the average of the three determinations being presented.

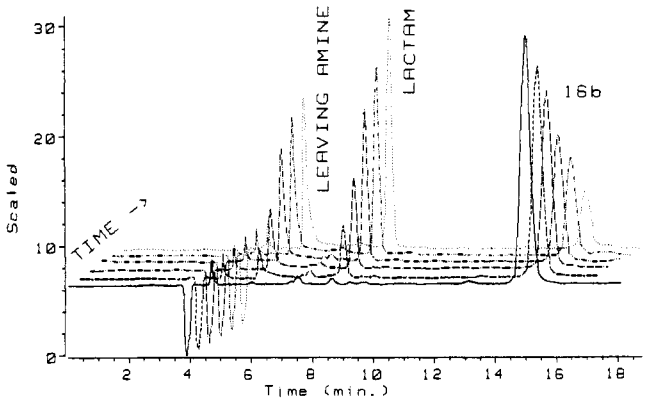


Figure 2. Representative series of chromatograms showing the separation of the amino/amide **16b** from the lactam and *p*-dimethylaminoaniline in the reaction mixture with time.

creasing pH (Figure 1), the cyclization being subject to general catalysis by acidic buffer components, obeying the equation $k_0 = k_{H_3O^+}[H_3O^+] + k_{H_2O}$. Over the pH range studied, the amount of free amine present is not rate-determining.

Compounds **24b** and **25b** are analogues of **12b** where an electron-withdrawing group (N=, σ_m = ca. 0.7, and CONHR, σ_p = 0.36, respectively) is present in the amine-bearing ring. Such electron-withdrawing substituents will ultimately be necessary to raise the reduction potential of the nitro group in the corresponding prodrugs. As expected, these substituents slowed the rate of cyclization, but the effects were modest at pH 6.8 ((8–10)-fold slower). The very slow cyclization of **24b** at pH 2.4 is consistent with protonation of the pyridyl nitrogen at this pH. Replacement of CH_2 (σ_m = −0.07) with SO_2 (σ_m = 0.60) slows cyclization by a much greater margin (cf. compounds **9b**,**10b** with **21b**,**22b**), but in this case, the possibility of quite different minimum-energy geometries for the compounds cannot be discounted (see below).

Previous work on the 2′-hydroxyphenylpropanamide **6** and analogues[14] and on 2′-hydroxyphenylpropanoic acids[15] has shown that 6′-methyl substitution greatly enhances the rate of internal cyclizations, but only when the linker chain possesses 3,3-dimethyl substitution. Similarly, in a study of a series of 5- and 7-substituted 4,4-dimethyl-6-hydroxyhydrocoumarins, the rate of lactonization was found to increase with the size of the C-5 substituent.[16]

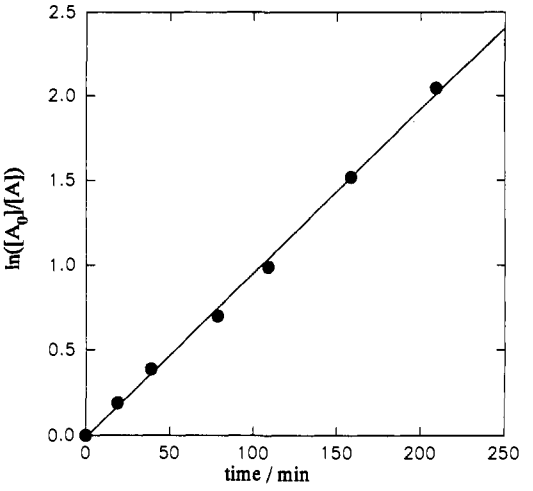
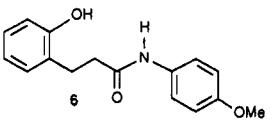


Figure 3. Representative plot showing the first-order loss of peak area of **16b**, where $[A]_0$ is the peak area of **16b** at time = zero and $[A]$ is the peak area at time t.



The only example of a 6′-substituent in the present study is compound **23b**, the 6′-methyl analogue of **21b**, whose observed pseudo-first-order rate constant was measured as 4.0×10^{-4} min^{-1} at pH 2.4, 37 °C. The rate of cyclization appears to be enhanced by the presence of the 6′-methyl substituent, by comparison with the observed stability of **21b** under identical conditions. However, in all of these cases, the electronic effects of the 6′-group on the amine cannot be divorced from their effects on molecular conformation (see below).

It should be noted that reductive cyclization of the nitro amides **7a–31a** does not have to proceed via the amines but may occur directly from the hydroxylamines. Entwistle[12] observed 90% loss of *N*-(3-quinolyl)-3-(2-nitrophenyl)propanamide (**10a**) on refluxing in cyclohexene in the presence of 10% Pd–C catalyst and attributed this to cyclization via the amine. However, the reduction of *m*-dinitrobenzene using this reagent has been shown to proceed via the hydroxylamine as an intermediate.[17] In the present work, the amines were isolated prior to studying their subsequent cyclization and, in contrast to the rapid reaction reported by Entwistle, *N*-(3-quinolyl)-3-(2-aminophenyl)propanamide (**10b**) was found to be quite stable under near-physiological conditions. Thus, it is possible that the more rapid cyclization observed by Entwistle reflected cyclization via the hydroxylamine. The reductive cyclization of ethyl 3-methyl-5-(2-nitrophenyl)-1-phenylpyrazole-4-carboxylate (analogous to **30a**) has been shown to proceed via either the hydroxylamine or the amine, depending on the conditions employed,[18] as determined by the relative yields of N-hydroxylactam and lactam. The isolated amine **30b** cyclized relatively slowly under the present conditions, by comparison with some of the other compounds studied.

Effects of Variation in the Link Group. Changes in the geometry of the link group between the amine-bearing ring and the leaving group can greatly influence the rate of formation of the tetrahedral intermediate **III** by two processes.[19] Restriction of rotational freedom of the ground-state conformation of the molecule can place the amine in a more favorable position with respect to the amide, a concept termed "stereopopulation control".[15,19]

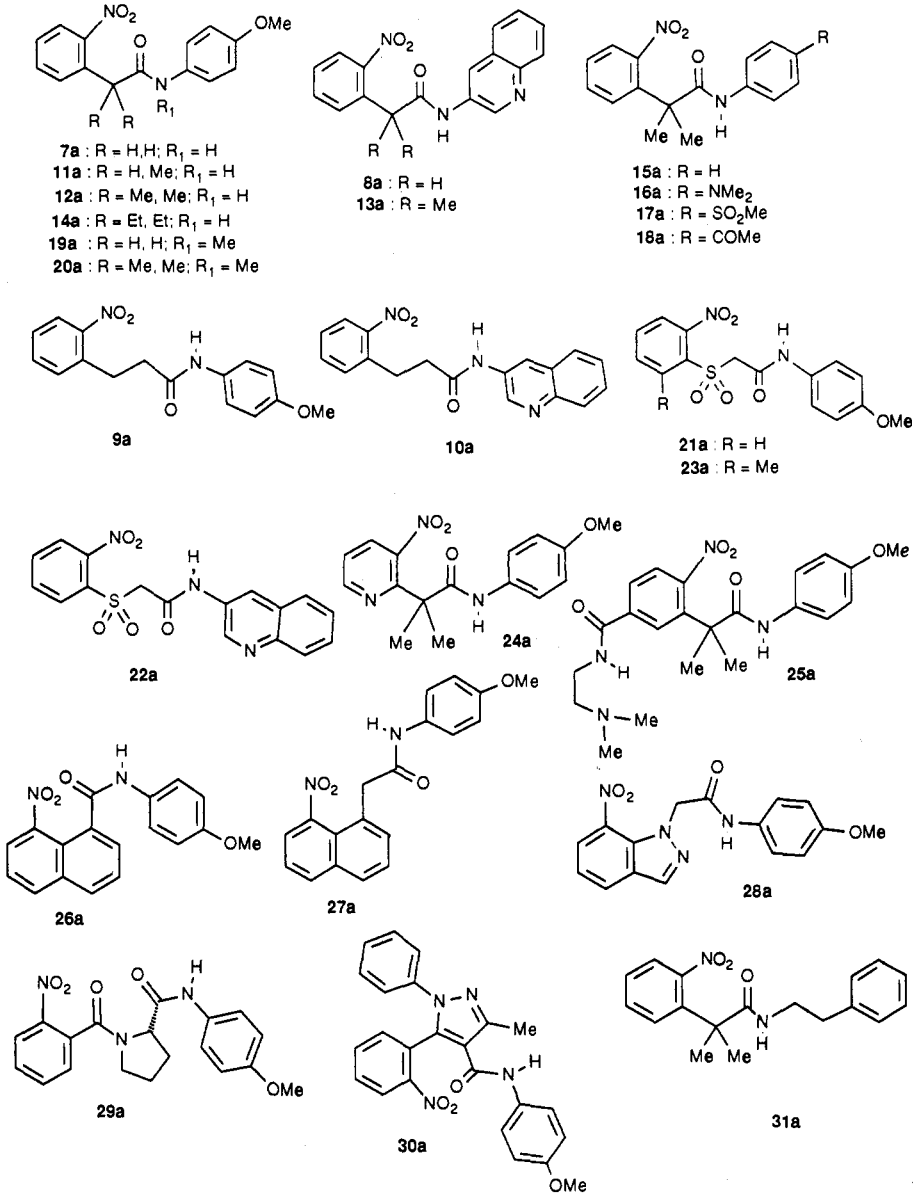


Figure 4. Structures of 2-nitro amides **7a–31a**.

In addition to this, the relief of steric compression which can accompany cyclization also greatly enhances reaction rates.[19] These aspects have been clearly demonstrated previously by the "trimethyl lock" effect, a pattern of methyl substitution which has been found to enhance enormously the rate of lactonization of compounds such as 3-(2-hydroxyphenyl)propionic acids[15] and 3-(2-hydroxyphenyl)propanamides.[14] Thus, while 3,3-dimethyl substitution of the 2′-hydroxyphenylpropanamide **6** results in a rate increase of 400-fold, addition of a 6′-methyl group provides an additional increase in rate of 90-fold.[14] In the present work, the same effect is reflected in the increasing relative rates of cyclization of the analogous unsubstituted (**7b**), monomethyl (**11b**), and *gem*-dimethyl (**12b**) amines. It can be seen from Figure 1 that the pH dependencies of the compounds run approximately parallel to each other and that, under these buffer conditions, the relative rates of cyclization are 1:26:100 and 1:8:500 (at pH values 2.4 and 6.8, respectively).

The relative rates at pH 2.4 obtained from the pH–rate profile contain an error due to lack of correction for amine protonation of **12b**, which leads to an underestimation of the rate of cyclization for this compound. However, the amine pK_a values are such that the relative rates will be unaffected by protonation at pH 6.8. Consideration of the rate enhancement of 400-fold observed in the 2′-hydroxyphenylpropanamide series[14] suggests that the stereochemical effects of *gem*-methyl substitution are comparable in both systems. The corresponding trimethyl-locked amine **54** has not been prepared but can be predicted to have a rate constant approximately 90-fold larger than **12b**, with a corresponding half-life of 48 s at pH 6.8. The more bulky *gem*-diethyl link group found in **14b** decreased the rate of cyclization of this compound relative to that of **12b** (although it still cyclizes significantly faster than the unsubstituted parent **7b**).

In light of these results, the low rates of cyclization of the sulfonyl compounds **21b** and **22b**, of similar topology to **12b**, are interesting. If the oxygen atoms of the sulfonyl provide a similar degree of preorganization as do the methyl groups of **12b**, one might have expected a similar improvement in reactivity on steric grounds, especially for the 6′-methyl derivative **23b**, which is a close mimic of the trimethyl lock geometry.[14] In fact, **21b–23b** proved among the most unreactive of all the compounds studied; the relative influences of the competing steric (activating) and electronic (deactivating) effects cannot be decided at this time.

Phenyl rings fused to the linker carbons have been considered as full analogues of the trimethyl lock because of the conformational restrictions imposed. In a previous investigation of steric rate acceleration of the lactonization of 2′-hydroxyphenylpropanoic acids,[19] an increase in rate of 2.1×10^4 was seen on going to the conformationally-locked analogue 8-hydroxynaphthalene-1-acetic acid. In the present study, an increased rate of cyclization is observed between the propanamide **9b** and its totally-locked analogue, the naphthalene-1-acetamide **27b**, although this could not be quantified because the latter compound cyclized spontaneously during the nitro-reduction step.

No rate enhancement was observed at pH 2.4 between propanamide **9b** and the indazoleacetamide **28b**, which is similarly conformationally restrained. Compound **30b** is another example of a compound possessing steric bulk in the linker portion of the molecule and which upon cyclization forms a six-membered ring. This compound displays only a minimal enhancement in cyclization rate at pH 2.4 over its unsubstituted counterpart **9b**. Compound **29b** which forms a seven-membered ring is less reactive again.

A moderate rate enhancement (42-fold) at pH 2.4 is observed between compound **7b** and its totally-locked analogue, the naphthalenecarboxamide **26b**. This lower-than-expected difference may arise from several contributing effects. Firstly, a five-membered rather than a six-membered[15] ring is being formed. Secondly, the more electron-deficient ring system of **26b** may also slow the reaction. Thirdly, the diaromatic amide link in **26b** is chemically more stable than that in **7b**.

Changes in the link group can also conceivably affect the rate of formation and breakdown of the tetrahedral intermediate, both of which have been proposed as the rate-determining step in some cases.[16,20,21] Compounds **19b** and **20b** were studied in order to observe the effects of methyl substitution of the amidic nitrogen. There is evidence[22] that severe distortion of the amidic unit does accelerate the rate of hydrolysis of amides, as the kinetic pK_a of the amide increases with distortion along with both acid- and base-catalyzed hydrolysis. However, in the present case, there was a significant decrease in the rates of cyclization of the *N*-methyl amides **19b** and **20b** compared with those of the unsubstituted analogues (**7b** and **12b**, respectively). The extent of amidic distortion on methyl substitution on these compounds is likely to be small and is apparently outweighed by other factors, most likely steric inhibition of nucleophilic attack or breakdown of the tetrahedral intermediate. The geometry of the forming lactam may also influence cyclization rates. Among compounds with fully flexible linker chains, the propionic acid analogues, which form a six-membered lactam (e.g., compounds **9b** and **10b**) cyclize more rapidly at pH 2.4 than do the corresponding acetic acid analogues (**7b** and **8b**). The former compounds are also likely to be stronger amines (by 1–2 pK_a units) than the latter.

Effects of Variation in the Leaving Group. The results in Table 1 show that the nature of the leaving group has an effect on the rate of cyclization. Thus, the 3-quinolyl compounds cyclize more rapidly than the corresponding 4-methoxyanilines (cf. **7b** with **8b** and **12b** with **13b**, respectively, at pH 2.4). (A rate constant could not be determined for **13b**, which cyclized spontaneously during the reduction process.) Additionally, cyclization of the aliphatic amide **31b** was very rapid, occurring spontaneously during the reduction process (Table 1). A previous study,[23] looking at substituted aniline release from hydroxy amide intermediates, has suggested that the effects of the leaving group on the rates of cyclization are small, with an observed rate enhancement factor of approximately 1.2 between *p*-methoxyaniline and aniline. However, this work was carried out on a system containing a full trimethyl lock, the stereochemical effects of which are so marked that they may obscure effects due to electronic changes in the leaving amine. In the present study of 2-aminoaryl amides, the rate enhancement factor between the corresponding compounds **12b** and **15b** was larger (~2-fold at both pH 2.4 and 6.8, at constant ionic strength).

A fuller investigation was therefore carried out on the effect on the rate of cyclization of varying electronic properties of the leaving group, using a series of 4-substituted analogues **12b**, **15b**, **16b**, and **18b**. The very slow reduction of the SO_2Me-substituted compound **17a** precluded inclusion of the corresponding amine **17b**. Within this series (although data at pH 2.4 could not be corrected for the effect of amine protonation), the electronic effects of the leaving group substitutions are expected to be isolated from the ring bearing the amine and not affect the pK_a of the amine within the series. This is substantiated by the mirroring of the trend at the two pH values (with the exception of **16b**).

However, comparison of the rate data for these compounds shows that, over an extended range of σ values, the effects of σ on k_{obs} are much smaller than expected from the original comparison of **12b** and **15b** (the very rapid cyclization of **16b** at pH 2.4 may arise from protonation of the dimethylamino group). More detailed studies are in progress with selected compounds to determine the rate constants for the buffer-, hydroxide-, and water-catalyzed components of the overall cyclization reaction (represented by k_{obs}) to elucidate further the leaving-group effect.

Conclusions

These preliminary investigations have investigated three factors which influence the overall rate of cyclization of (2-aminophenyl)alkanamides: the nucleophilicity of the amine, the geometry of the compound (as determined by the nature of the linking group between the amine and carbonyl groups), and the nature of the leaving group. The way in which these influence the cyclization depends on whether formation or breakdown of the tetrahedral intermediate **III** is rate-determining.

In the lactonization of both 3-(2-hydroxyphenyl)propanoic acids[15] and the propanamide **6**,[14] the rate-determining step is proposed to be breakdown of the tetrahedral intermediate. These cases are different from the present one in that a hydroxy group rather than an amine is the nucleophilic species. In the present work, the pH dependence of the reaction shows no evidence of any change in the rate-determining step over the pH range studied. The observed cyclization rates are consistent with rate-determining formation of the tetrahedral intermediate, where factors such as amine nucleophilicity, compound stereochemistry, and alkyl chain length would directly influence the ring-forming equilibria prior to the rate-determining step, while the leaving group is expected to have minimal influence upon the rate of formation of the tetrahedral intermediate.

These model studies suggest that phenyldimethylacetamides could be suitable prodrugs for the bioreductively-

triggered release of amines, with **12b** having a half-life for cyclization of 72 min at near-physiological pH (6.8). As it stands, this is too slow to be useful, but further significant rate enhancements appear possible by alterations in the geometry of the system (e.g., by using the trimethyl lock). While substitution of electron-withdrawing groups at R_1 (Scheme 1) will be required to raise the nitro-group reduction potential into the appropriate range, the concomitant slowdown in rates of cyclization (at pH 6.8) appears to be modest. Future work in this series will clarify the role of the leaving group, evaluate the nature of catalysis of the cyclization, and investigate the kinetics of cyclization via intermediate reduction products. Several of the more rigid systems are also worthy of further attention; in particular, the naphthaleneacetamide **27a** cyclized spontaneously during nitro reduction. Whether the corresponding amine **27b** cyclizes very rapidly, or whether putative reduction intermediates such as the hydroxylamine undergo this rapid cyclization, remains to be determined.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical and were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were measured on a Bruker AM-400 spectrometer (Me_4Si).

Preparation of Nitro Acids. The following compounds were prepared by literature methods and had physical constants consistent with those reported: 3-(2-nitrophenyl)propanoic acid,[24] 8-nitronaphthalene-1-carboxylic acid,[25] 2-(2-nitrophenyl)propanoic acid,[26] [(2-nitrophenyl)sulfonyl]acetic acid,[27] (2*S*)-*N*-(2-nitrobenzoyl)proline,[28] and 3-methyl-5-(2-nitrophenyl)-1-phenylpyrazole-4-carboxylic acid.[29]

2-Methyl-2-(2-nitrophenyl)propanoic Acid (40) and 2-Ethyl-2-(2-nitrophenyl)butanoic Acid (41). A solution of methyl 2-methyl-2-(2-nitrophenyl)propanoate[8] (6.6 g, 0.03 mol) in concentrated H_2SO_4 (40 mL) was added dropwise to vigorously stirred crushed ice (300 g). The precipitated solid was collected, washed with water, and partitioned between aqueous $KHCO_3$ and CH_2Cl_2. The aqueous layer was washed twice with CH_2Cl_2, clarified by filtration, and acidified with HCl to give 2-methyl-2-(2-nitrophenyl)propanoic acid (**40**) (70% yield), mp (water 148–149 °C: ^{1}H NMR ($CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 1 H, H-3), 7.59–7.65 (m, 2 H, H-5,6), 7.42 (pent, *J* = 4.2 Hz, 1 H, H-4), 1.70 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{10}H_{11}NO_4$) C,H,N. Similar hydrolysis of methyl 2-ethyl-2-(2-nitrophenyl)butanoate[8] gave 2-ethyl-2-(2-nitrophenyl)butanoic acid (**41**) (71% yield), mp (benzene/petroleum ether) 150–150.5 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 12.03 (br s, 1 H, COOH), 7.85 (dd, *J* = 8.0, 1.4 Hz, 1 H, H-3), 7.69 (td, *J* = 7.6, 1.4 Hz, 1 H, H-5), 7.60 (dd, *J* = 8.0, 1.2 Hz, 1 H, H-6), 7.56 (td, *J* = 7.6, 1.2 Hz, 1 H, H-4), 2.07 (qd, *J* = 7.5, 1.9 Hz, 4 H, 2 × CH_2), 0.69 (t, *J* = 7.5 Hz, 6 H, 2 × CH_3). Anal. ($C_{12}H_{15}NO_4$) C,H,N.

[(2-Methyl-6-nitrophenyl)sulfonyl]acetic Acid (39). Via a published general method[30] for the synthesis of 2-nitrobenzenethiols, powdered $Na_2S{\cdot}xH_2O$ (60% Na_2S, 7.6 g, 58 mmol) was added to a solution of 1-methyl-2-chloro-3-nitrobenzene (**36**) (10.0 g, 58 mmol) in DMSO (180 mL). The mixture was stirred at 25 °C for 24 h and then diluted with water (1.2 L), clarified by filtration, acidified with HCl, and extracted with toluene (2 × 400 mL). The residue from evaporation of the combined toluene extracts was chromatographed on silica gel. Elution with petroleum ether/CH_2Cl_2 (13:7) gave 2-methyl-6-nitrobenzenethiol (**37**) (4.94 g, 50%), mp (petroleum ether) 135–137 °C: ^{1}H NMR ($CDCl_3$) δ 7.98 (d, *J* = 8.3 Hz, 1 H, H-5), 7.44 (d, *J* = 7.4 Hz, 1 H, H-3), 7.71 (t, *J* = 7.9 Hz, 1 H, H-4), 4.61 (s, 1 H, SH), 2.47 (s, 3 H, CH_3). A solution of **37** (2.05 g, 12 mmol) in 2.0 N aqueous NaOH (13.2 mL, 26 mmol) was treated with chloroacetic acid (1.32 g, 14 mmol). The mixture was stirred at 20 °C for 15 min and then at 90 °C for 15 min, cooled, treated with charcoal, clarified by filtration, and acidified with HCl. The resulting solid was dried and crystallized from benzene/petroleum ether to give [(2-methyl-6-nitrophenyl)thio]acetic acid (**38**) (1.60 g, 58%), mp 105–106 °C: ^{1}H NMR ($CDCl_3$) δ 7.46–7.50 (m, 2 H, H-3,5), 7.40 (dd, *J* = 8.1, 7.4 Hz, 1 H, H-4), 6.3 (br s, 1 H, COOH), 3.56 (s, 2 H, CH_2), 2.64 (s, 3 H, CH_3). Anal. ($C_9H_9NO_4S$) C,H,N. The thioacetic acid **38** (3.18 g, 14 mmol) in AcOH (24 mL) containing 30% H_2O_2 (9 mL) was heated at 80 °C for 3 h, after which time additional H_2O_2 (9 mL) was added and the heating continued for a further 3 h (lower reaction temperatures gave predominantly the corresponding sulfinylacetic acid). The mixture was evaporated to dryness under reduced pressure, and the residue was crystallized successively from water and benzene to give [(2-methyl-6-nitrophenyl)sulfonyl]acetic acid (**39**) (2.85 g, 79%), mp 145–146 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 13.6 (br s, 1 H, COOH), 7.78–7.86 (m, 2 H, H-3,4), 7.74 (dd, *J* = 6.9, 2.0 Hz, 1 H, H-5), 4.59 (s, 2 H, CH_2), 2.72 (s, 3 H, CH_3). Anal. ($C_9H_9NO_6S$) C,H,N.

7-Nitroindazole-1-acetic Acid (35). A mixture of 7-nitroindazole (**32**) (2.61 g, 16 mmol) and powdered K_2CO_3 (3.30 g, 24 mmol) in DMF (20 mL) was treated with ethyl bromoacetate (3.01 g, 18 mmol) and then stirred at 20 °C for 6 h. The mixture was filtered, the volatiles were removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was worked-up and the residue chromatographed on silica gel, elution with petroleum ether/CH_2Cl_2 (1:5), giving ethyl 7-nitroindazole-1-acetate (**33**) (0.91 g, 23%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.23 (dd, *J* = 7.8, 1.0 Hz, 1 H, H-6), 8.21 (s, 1 H, H-3), 8.07 (dd, *J* = 7.9, 1.0 Hz, 1 H, H-4), 7.28 (t, *J* = 7.9 Hz, 1 H, H-5), 5.57 (s, 2 H, NCH_2), 4.20 (q, *J* = 7.1 Hz, 2 H, CH_2CH_3), 1.26 (t, *J* = 7.1 Hz, 3 H, CH_3). Anal. ($C_{11}H_{11}N_3O_4$) C,H,N. Basic hydrolysis of this acetate gave 7-nitroindazole-1-acetic acid (**35**) (84% yield), mp (aqueous MeOH) 217–218 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 8.45 (s, 1 H, H-3), 8.29 (dd, *J* = 7.9, 1.0 Hz, 1 H, H-6), 8.25 (dd, *J* = 7.8, 1.0 Hz, 1 H, H-4), 7.38 (t, *J* = 7.8 Hz, 1 H, H-5), 5.44 (s, 2 H, CH_2). Anal. ($C_9H_7N_3O_4$) C,H,N. Further elution of the original column with CH_2Cl_2 gave ethyl 7-nitroindazole-2-acetate (**34** (0.76 g, 19%), mp (benzene) 135–137 °C: ^{1}H NMR ($CDCl_3$) δ 8.37 (dd, *J* = 7.6, 0.9 Hz, 1 H, H-6), 8.34 (s, 1 H, H-3), 8.08 (dd, *J* = 8.3, 0.9 Hz, 1 H, H-4), 7.22 (dd, *J* = 8.2, 7.6 Hz, 1 H, H-5), 5.38 (s, 2 H, NCH_2), 4.28 (q, *J* = 7.1 Hz, 2 H, CH_2CH_3), 1.31 (t, *J* = 7.1 Hz, 3 H, CH_3). Anal. ($C_{11}H_{11}N_3O_4$) C,H,N.

8-Nitronaphthalene-1-acetic Acid (42). A two-phase mixture of 1-(bromomethyl)-8-nitronaphthalene[9] (4.0 g, 15 mmol), NaCN (2.2 g, 45 mmol), and tetrabutylammonium bromide (0.48 g, 1.5 mmol) in CH_2Cl_2 (20 mL) and water (20 mL) was stirred vigorously at 20 °C for 36 h. Workup of the organic layer and chromatography of the product on alumina, eluting with petroleum ether/CH_2Cl_2 (1:1), gave 8-nitronaphthalene-1-acetonitrile (0.92 g, 29%) as light-sensitive needles, mp (petroleum ether) 102 °C: ^{1}H NMR ($CDCl_3$) δ 8.12 (dd, *J* = 8.3, 1.2 Hz, 1 H, H-7), 7.94–8.01 (m, 2 H, H-2,4), 7.84 (dd, *J* = 7.4, 1.1 Hz, 1 H, H-5), 7.55–7.70 (m, 2 H, H-3,6), 3.99 (s, 2 H, CH_2). Anal. ($C_{12}H_8N_2O_2$) C,H,N. The nitrile (0.63 g) was hydrolyzed by heating under reflux in a mixture of AcOH/concentrated H_2SO_4/H_2O (1:1:1, 10 mL) for 1 h. The cooled solution was diluted with ice-cold NH_4OH, filtered, and acidified with concentrated HCl to give the light-sensitive 8-nitronaphthalene-1-acetic acid (**42**) (0.51 g, 74%), mp (benzene/MeOH) 196–198 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 8.29 (d, *J* = 8.2 Hz, 1 H, H-7), 8.10 (dd, *J* = 7.6, 1.8 Hz, 1 H, H-4), 7.97 (dd, *J* = 7.4, 1.0 Hz, 1 H, H-5), 7.60–7.70 (m, 3 H, H-2,3,6), 3.85 (s, 2 H, CH_2). Anal. ($C_{12}H_9NO_4$) C,H,N.

2-Methyl-2-[2-(3-nitropyridyl)]propanoic Acid (46). Diethyl [2-(3-nitropyridyl)]malonate (**43**) was prepared as described,[31] purified by chromatography on silica gel (elution with CH_2Cl_2), and crystallized from diisopropyl ether, mp 61–62 °C (lit.[31] oil): ^{1}H NMR ($CDCl_3$) δ 8.83 (dd, *J* = 4.7, 1.6 Hz, 1 H, H-6), 8.49 (dd, *J* = 8.3, 1.6 Hz, 1 H, H-4), 7.53 (q, *J* = 8.3, 4.7 Hz, 1 H, H-5), 5.53 (s, 1 H, CH), 4.31 (2 q, *J* = 7.1 Hz, 4 H, 2 × CH_2CH_3), 1.30 (t, *J* = 7.1 Hz, 6 H, 2 × CH_3).

A solution of **43** (6.0 g, 21 mmol) in dry DMSO (20 mL) containing water (0.76 g, 42 mmol) was stirred at 150 °C under an efficient condenser for 3 h.[32] Excess solvent was then removed under reduced pressure, the residue was dissolved in CH_2Cl_2, and the resulting solution was washed twice with water. Evaporation and chromatography of the residue on silica gel, eluting

with CH_2Cl_2/MeOH (49:1), gave ethyl [2-(3-nitropyridyl)]acetate (**44**) (3.22 g, 72%) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.80 (dd, J = 4.7, 1.5 Hz, 1 H, H-6), 8.43 (dd, J = 8.3, 1.5 Hz, 1 H, H-4), 7.48 (dd, J = 8.3, 4.7 Hz, 1 H, H-5), 4.33 (s, 2 H, CH_2COO), 4.20 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 1.26 (t, J = 7.1 Hz, 3 H, CH_3). Anal. ($C_9H_{10}N_2O_4$) C,H,N.

A stirred mixture of **44** (1.68 g, 8 mmol), freshly distilled MeI (2.49 g, 17.5 mmol), and 18-crown-6 (0.21 g, 0.8 mmol) in dry DMF (12 mL) was treated portionwise with NaH (0.70 g, 17.5 mmol, as a 60% dispersion in mineral oil) over 10 min, while the temperature was maintained below 5 °C with external cooling. The mixture was stirred at 5 °C for a further 1 h and then at 20 °C for 1 h before being diluted with CH_2Cl_2 (50 mL). The organic layer was washed successively with dilute AcOH, aqueous Na_2CO_3, and water (3×). Evaporation gave a residue which was chromatographed on silica gel. Elution with petroleum ether removed mineral oil and impurities, and further elution with petroleum ether/CH_2Cl_2 (3:7) gave ethyl 2-methyl-2-[2-(3-nitropyridyl)]propanoate (**45**) (1.62 g, 85%) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.79 (dd, J = 4.7, 1.6 Hz, 1 H, H-6), 8.24 (dd, J = 8.2, 1.6 Hz, 1 H, H-4), 7.40 (dd, J = 8.2, 4.7 Hz, 1 H, H-5), 4.12 (q, J = 7.1 Hz, 2 H, CH_2), 1.72 (s, 6 H, $C(CH_3)_2$), 1.20 (t, J = 7.1 Hz, 3 H, CH_2CH_3). Anal. ($C_{11}H_{14}N_2O_4$) C,H,N.

The above ester (**45**) (2.0 g) was heated at 80 °C in concentrated H_2SO_4 (8 mL) for 4 h and then cooled, basified with ice/concentrated NH_4OH, and clarified by filtration. Acidification with HCl followed by prolonged cooling gave a precipitate which was collected, washed with cold saturated NaCl, and crystallized from benzene/petroleum ether to give 2-methyl-2-[2-(3-nitropyridyl)]propanoic acid (**46**) (68% yield), mp 111 °C: ^{1}H NMR ($CDCl_3$) δ 8.80 (dd, J = 4.7, 1.6 Hz, 1 H, H-6), 8.29 (dd, J = 8.1, 1.6 Hz, 1 H, H-4), 7.42 (dd, J = 8.1, 4.7 Hz, 1 H, H-5), 1.76 (s, 6 H, $C(CH_3)_2$). Anal. ($C_9H_{10}N_2O_4$) C,H,N.

***N*-(4-Methoxyphenyl)-2-(2-nitrophenyl)propanamide (11a): Example of Amide Synthesis A.** A stirred solution of 2-(2-nitrophenyl)propanoic acid (600 mg, 3.08 mmol), 4-methoxyaniline (397 mg, 3.23 mmol), and 1-methylimidazole (303 mg, 3.70 mmol) in DMF (4 mL) was treated dropwise at 0 °C with diethyl cyanophosphonate (98%, 564 mg, 3.39 mmol). The mixture was kept at 20 °C for 24 h, and then concentrated under reduced pressure. The residue was shaken with dilute aqueous Na_2CO_3, and the resulting solid was crystallized from aqueous MeOH to give *N*-(4-methoxyphenyl)-2-(2-nitrophenyl)propanamide (**11a**) (592 mg, 64%), mp 139–140 °C: ^{1}H NMR ($CDCl_3$) δ 7.90 (br s, 1 H, CONH), 7.83 (dd, J = 8.2, 1.3 Hz, 1 H, H-3), 7.74 (dd, J = 8.0, 1.2 Hz, 1 H, H-6), 7.62 (td, J = 7.7, 1.3 Hz, 1 H, H-5), 7.37–7.44 (m, 1 H, H-4), 7.39 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.82 (d, J = 9.0 Hz, 2 H, H-3′,5′), 4.25 (q, J = 6.9 Hz, CH), 3.77 (s, 3 H, OCH_3), 1.58 (d, J = 6.9 Hz, 3 H, $CHCH_3$). Anal. ($C_{16}H_{16}N_2O_4$) C,H,N. Similarly were prepared the following compounds:

***N*-(4-Methoxyphenyl)-2-nitrophenylacetamide (7a)**, mp 192–194 °C. (lit.[33] mp not given).

***N*-(3-Quinolyl)-2-nitrophenylacetamide (8a)**, mp (aqueous MeOH) 202–203 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 10.75 (s, 1 H, CONH), 8.93 (d, J = 2.5 Hz, 1-H, H-2′), 8.64 (d, J = 2.5 Hz, 1 H, H-4′), 8.11 (dd, J = 7.5, 1.8 Hz, 1 H, H-3), 7.53–7.96 (m, 7 H, H-4,5,6,5′,6′,7′,8′), 4.25 (s, 2 H, CH_2). Anal. ($C_{17}H_{13}N_3O_3$) C,H,N.

***N*-[4-(Methylcarbonyl)phenyl]-2-methyl-2-(2-nitrophenyl)propanamide (18a)**, mp (aqueous MeOH) 154 °C: ^{1}H NMR ($CDCl_3$) 7.90 (d, J = 8.8 Hz, 2 H, H-2′,6′), 7.85–7.91 (m, 1 H, H-3), 7.64–7.73 (m, 2 H, H-5,6), 7.54 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.49 (m, 1 H, H-4), 7.29 (br s, 1 H, CONH), 2.56 (s, 3 H, $COCH_3$), 1.77 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{18}N_2O_4$) C,H,N.

***N*-(4-Methoxyphenyl)-*N*-methyl-2-nitrophenylacetamide (19a)**, mp (aqueous MeOH) 92 °C: ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 8.1 Hz, 1 H, H-3), 7.53 (t, J = 7.5 Hz, 1 H, H-5), 7.40 (t, J = 7.5 Hz, 1 H, H-4), 7.22–7.30 (m, 3 H, H-6,2′,6′), 6.97 (d, J = 8.8 Hz, 2 H, H-3′,5′), 3.84 (s, 3 H, OCH_3), 3.75 (s, 2 H, CH_2CO), 3.26 (s, 3 H, NCH_3). Anal. ($C_{18}H_{18}N_2O_4$) C,H,N.

***N*-(4-Methoxyphenyl)-[(2-nitrophenyl)sulfonyl]acetamide (21a)**, mp (aqueous MeOH) 211–212 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 10.35 (s, 1 H, CONH), 8.12 (dd, J = 7.8, 1.3 Hz, 1 H, H-3), 8.08 (dd, J = 7.7, 1.5 Hz, 1 H, H-6), 8.02 (td, J = 7.7, 1.5 Hz, 1 H, H-4), 7.96 (td, J = 7.6, 1.3 Hz, 1 H, H-5), 7.42 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.90 (d, J = 9.0 Hz, 2 H, H-3′,5′), 4.71 (s, 2 H, CH_2CONH), 3.72 (s, 3 H, OCH_3). Anal. ($C_{15}H_{14}N_2O_6S$) C,H,N.

***N*-(3-Quinolyl)-[(2-nitrophenyl)sulfonyl]acetamide (22a)**, mp (MeOH) 107–110 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 11.01 (s, 1 H, CONH), 8.86 (d, J = 2.4 Hz, 1 H, H-2′), 8.64 (d, J = 2.4 Hz, 1 H, H-4′), 8.11–8.19 (m, 2 H, H-3,6), 7.92–8.08 (m, 4 H, H-4,5,5′,8′), 7.55–7.73 (m, 2 H, H-6′,7′), 4.88 (s, 2 H, CH_2). Anal. ($C_{17}H_{13}N_3O_5S$) C,H,N.

***N*-(4-Methoxyphenyl)-[(2-methyl-6-nitrophenyl)sulfonyl]acetamide (23a)**, mp (aqueous MeOH) 209–211 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 10.42 (s, 1 H, CONH), 7.78–7.87 (m, 2 H, H-4,5), 7.73 (dd, J = 6.9, 2.2 Hz, 1 H, H-3), 7.47 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.92 (d, J = 9.0 Hz, 2 H, H-3′,5′), 4.56 (s, 2 H, CH_2), 3.73 (s, 3 H, OCH_3), 2.67 (s, 3 H, CH_3). Anal. ($C_{16}H_{16}N_2O_6S \cdot 0.5H_2O$) C,H,N.

***N*-(4-Methoxyphenyl)-8-nitronaphthalene-1-carboxamide (26a)**, mp (aqueous MeOH) 241–242 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 10.64 (s, 1 H, CONH), 8.41 (dd, J = 8.3, 0.9 Hz, 1 H, H-7), 8.29 (dd, J = 8.3, 0.8 Hz, 1 H, H-2), 8.19 (dd, J = 7.5, 1.1 Hz, 1 H, H-4 or H-5), 8.02 (dd, J = 7.2, 1.1 Hz, 1 H, H-4 or H-5), 7.72–7.84 (m, 2 H, H-3,6), 7.61 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.95 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.76 (s, 3 H, CH_3). Anal. ($C_{18}H_{14}N_2O_4$) C,H,N.

***N*-(4-Methoxyphenyl)-8-nitronaphthalene-1-acetamide (27a)**, mp (MeOH) 196–196.5 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 9.99 (s, 1 H, CONH), 8.29 (dd, J = 8.3, 1.0 Hz, 1 H, H-7), 8.09 (dd, J = 7.5, 1.9 Hz, 1 H, H-4), 7.98 (dd, J = 7.4, 1.1 Hz, 1 H, H-5), 7.62–7.71 (m, 3 H, H-2,3,6), 7.45 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.87 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.93 (s, 2 H, CH_2), 3.71 (s, 3 H, CH_3). Anal. ($C_{19}H_{16}N_2O_4$) C,H,N.

***N*-(4-Methoxyphenyl)-7-nitroindazole-1-acetamide (28a)**, mp (aqueous MeOH) 193 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 9.20 (s, 1 H, CONH), 8.46 (s, 1 H, H-3), 8.29 (dd, J = 8.0, 0.9 Hz, 1 H, H-6), 8.22 (dd, J = 7.7, 0.9 Hz, 1 H, H-4), 7.39 (d, J = 9.1 Hz, 2 H, H-2′,6′), 7.35–7.40 (m, 1 H, H-5), 6.89 (d, J = 9.1 Hz, 2 H, H-3′,5′), 5.53 (s, 2 H, CH_2CO), 3.71 (s, 3 H, OCH_3). Anal. ($C_{16}H_{14}N_4O_4$) C,H,N.

(2*S*)-*N*-(4-Methoxyphenyl)-1-(2-nitrobenzoyl)pyrrolidine-2-carboxamide (29a), mp (aqueous MeOH) 207–208 °C: ^{1}H NMR ($CDCl_3$) δ 9.16 (br s, 1 H, CONH), 8.23 (dd, J = 8.3, 0.9 Hz, 1 H, H-3), 7.76 (td, J = 7.5, 1.0 Hz, 1 H, H-5), 7.62 (td, J = 7.9, 1.4 Hz, 1 H, H-4), 7.47–7.55 (m, 1 H, H-6), 7.51 (d, J = 9.0 Hz, 2 H, H-2″,6″), 6.80 (d, J = 9.0 Hz, 2 H, H-3″,5″), 3.77 (s, 3 H, H-2′), 4.92–4.98 (m, 1 H, H-2′), 3.18–3.38 (m, 2 H, H-5′), 1.92–2.62 (m, 4 H, H-3′,4′). Anal. ($C_{18}H_{19}N_3O_5$) C,H,N.

***N*-(4-Methoxyphenyl)-3-methyl-5-(2-nitrophenyl)-1-phenylpyrazole-4-carboxamide (30a): Example of Amide Synthesis B.** A mixture of 3-methyl-5-(2-nitrophenyl)-1-phenylpyrazole-4-carboxylic acid[29] (614 mg, 1.9 mmol) and $SOCl_2$ (5 mL) containing DMF (1 small drop) was heated under reflux for 15 min. The solution was evaporated to dryness under reduced pressure below 30 °C, benzene (10 mL) was added, and the mixture was reevaporated. The residue was cooled to −5 °C and treated with an ice-cold solution of 4-methoxyaniline (246 mg, 2.0 mmol) in pyridine (4 mL). After stirring at 20 °C for 15 min, the mixture was diluted with 2 N aqueous Na_2CO_3. The resulting solid was collected by filtration and crystallized from aqueous MeOH to give *N*-(4-methoxyphenyl)-3-methyl-5-(2-nitrophenyl)-1-phenylpyrazole-4-carboxamide (**30a**) (640 mg, 79%), mp 182–183 °C: ^{1}H NMR ($CDCl_3$) δ 8.02 (dd, J = 8.0, 1.4 Hz, 1 H, H-3), 7.62 (td, J = 7.5, 1.6 Hz, 1 H, H-4), 7.57 (td, J = 7.8, 1.4 Hz, 1 H, H-5), 7.44 (td, J = 7.1, 1.7 Hz, 1 H, H-6), 7.15–7.30 (m, 8 H, NH, H-2′,3′,4′,5′,6′,2″,6″), 6.80 (d, J = 9.0 Hz, 2 H, H-3″,5″), 3.75 (s, 3 H, OCH_3), 2.65 (s, 3 H, CH_3). Anal. ($C_{24}H_{20}N_4O_4$) C,H,N. Similarly were prepared the following compounds:

***N*-(4-Methoxyphenyl)-3-(2-nitrophenyl)propanamide (9a)**, mp (aqueous MeOH) 88–89 °C: ^{1}H NMR ($CDCl_3$) δ 7.95 (dd, J = 8.2, 1.1 Hz, 1 H, H-3), 7.54 (td, J = 7.6, 1.2 Hz, 1 H, H-5), 7.47 (dd, J = 7.4, 1.4 Hz, 1 H, H-6), 7.37 (m, 1 H, H-4), 7.37 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.22 (br s, 1 H, CONH), 6.84 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.78 (s, 3 H, OCH_3), 3.30 (t, J = 7.8 Hz, 2 H, CH_2CH_2CONH), 2.72 (t, J = 7.6 Hz, 2 H, CH_2CONH). Anal. ($C_{16}H_{16}N_2O_4$) C,H,N.

***N*-(3-Quinolyl)-3-(2-nitrophenyl)propanamide (10a)**, mp (aqueous MeOH) 127–128 °C (lit.[12] mp 105–106 °C).

***N*-(4-Methoxyphenyl)-2-methyl-2-(2-nitrophenyl)propanamide (12a)**, mp (aqueous MeOH) 156–157 °C: ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, J = 8.0, 1.4 Hz, 1 H, H-3), 7.69 (dd, J = 8.0, 1.5 Hz, 1 H, H-6), 7.64 (td, J = 7.6, 1.4 Hz, 1 H, H-5), 7.46 (td, J = 7.6,

1:5 Hz, 1 H, H-4), 7.32 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.95 (br s, 1 H, CONH), 6.83 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.77 (s, 3 H, OCH_3), 1.75 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{17}H_{18}N_2O_4$) C,H,N.

N-(3-Quinolyl)-2-methyl-2-(2-nitrophenyl)propanamide (13a), mp (benzene/petroleum ether) 76–80 °C: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 2.3 Hz, 1 H, H-2′), 8.62 (s, 1 H, CONH), 7.40–8.01 (m, 9 H, ArH), 1.81 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{17}N_3O_3$) C,H,N.

N-(4-Methoxyphenyl)-2-ethyl-2-(2-nitrophenyl)butanamide (14a), purified by chromatography on silica gel, eluting with CH_2Cl_2, mp ($(iPr)_2O$) 102 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 9.02 (s, 1 H, CONH), 7.76 (dd, J = 8.0, 1.3 Hz, 1 H, H-3), 7.69 (td, J = 7.6, 1.3 Hz, 1 H, H-5), 7.63 (dd, J = 8.0, 1.3 Hz, 1 H, H-6), 7.51 (td, J = 7.6, 1.3 Hz, 1 H, H-4), 7.33 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.85 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.72 (s, 3 H, OCH_3), 2.17 (q, J = 7.4 Hz, 4 H, 2 × CH_2CH_3), 0.69 (t, J = 7.4 Hz, 6 H, 2 × CH_3). Anal. ($C_{19}H_{22}N_2O_4$) C,H,N.

N-phenyl-2-methyl-2-(2-nitrophenyl)propanamide (15a), mp (aqueous MeOH) 118–119 °C: ^{1}H NMR ($CDCl_3$) δ 7.86 (dd, J = 8.0, 1.3 Hz, 1 H, H-3), 7.70 (dd, J = 8.0, 1.5 Hz, 1 H, H-6), 7.65 (td, J = 8.0, 1.4 Hz, 1 H, H-5), 7.47 (td, J = 7.7, 1.5 Hz, 1 H, H-4), 7.40–7.48 (m, 2 H, H-2′,6′), 7.25–7.35 (m, 2 H, H-3′,5′), 7.09 (m, 1 H, H-4′), 7.06 (br s, 1 H, CONH), 1.76 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{18}N_2O_3$) C,H,N.

N-[4-(Dimethylamino)phenyl]-2-methyl-2-(2-nitrophenyl)propanamide (16a), mp (aqueous MeOH) 165–166 °C: ^{1}H NMR ($CDCl_3$) δ 7.83 (dd, J = 8.0, 1.4 Hz, 1 H, H-3), 7.69 (dd, J = 8.0, 1.4 Hz, 1 H, H-6), 7.63 (td, J = 7.6, 1.4 Hz, 1 H, H-5), 7.45 (td, J = 7.5, 1.5 Hz, 1 H, H-4), 7.26 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.90 (br s, 1 H, CONH), 6.68 (d, J = 9.0 Hz, 2 H, H-3′,5′), 2.90 (s, 6 H, $N(CH_3)_2$), 1.74 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{21}N_3O_3$) C,H,N.

N-[4-(Methylsulfonyl)phenyl]-2-methyl-2-(2-nitrophenyl)propanamide (17a), reaction time 24 h, mp (benzene) 195–196 °C: ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.0, 1.1 Hz, 1 H, H-3), 7.84 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.7–7.7 (m, 2 H, H-5,6), 7.64 (d, J = 8.8 Hz, 2 H, H-2′,6′), 7.51 (m, 1 H, H-4), 7.36 (br s, 1 H, CONH), 3.01 (s, 3 H, OCH_3), 1.78 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{17}H_{18}N_2O_5S$) C,H,N.

N-(4-Methoxyphenyl)-N-2-methyl-2-(2-nitrophenyl)propanamide (20a), purified by chromatography on silica gel, eluting with CH_2Cl_2/EtOAc (9:1), mp (benzene/petroleum ether) 122–123 °C: ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 5.9 Hz, 1 H, H-3), 7.15 (m, 2 H, aromatic), 6.84 (d, J = 7.7 Hz, 2 H, H-2′,6′), 6.75 (m, 1 H, aromatic), 6.48 (d, J = 7.7 Hz, 2 H, H-3′,5′), 3.72 (s, 3 H, OCH_3), 3.23 (s, 3 H, NCH_3), 1.51 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{20}N_2O_4$) C,H,N.

N-(4-Methoxyphenyl)-2-methyl-2-[2-(3-nitropyridyl)]propanamide (24a), mp (aqueous MeOH) 152–154 °C: ^{1}H NMR ($CDCl_3$) δ 8.79 (dd, J = 4.7, 1.5 Hz, 1 H, H-6), 8.15 (dd, J = 8.2, 1.5 Hz, 1 H, H-4), 7.40 (dd, J = 8.2, 4.7 Hz, 1 H, H-5), 7.35 (d, J = 8.9 Hz, 2 H, H-2′,6′), 7.26 (br s, 1 H, NH), 6.85 (d, J = 8.9 Hz, 2 H, H-3′,5′), 3.78 (s, 3 H, CH_3), 1.81 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{17}N_3O_4$) C,H,N.

N-(2-Phenylethyl)-2-methyl-2-(2-nitrophenyl)propanamide (31a), purified by chromatography on silica gel, eluting with CH_2Cl_2/EtOAc (4:1), mp (benzene/petroleum ether) 89 °C: ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 8.0 Hz, 1 H, H-3), 7.53–7.60 (m, 2 H, H-5,6), 7.35–7.44 (m, 1 H, Hh4), 7.12–7.28 (m, 5 H, H-2′,3′,4′,5′,6), 5.42 (br s, 1 H, CONH), 3.50 (q, J = 6.6 Hz, 2 H, $NHCH_2CH_2$), 2.81 (t, J = 6.9 Hz, 2 H, $NHCH_2CH_2$), 1.59 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{20}N_2O_3$) C,H,N.

N-(4-Methoxyphenyl)-2-[5-[[2-(dimethylamino)ethyl]carbamoyl]-2-nitrophenyl]-2-methylpropanamide (25a). A mixture of 5-(methoxycarbonyl)-2-nitrobenzoic acid[34] (47) (25.0 g, 0.11 mmol), $SOCl_2$ (100 mL), and DMF (0.2 mL) was heated under reflux for 45 min. Excess reagent was removed under reduced pressure at below 30 °C, and the residue was dissolved in benzene and reevaporated. A solution of the residue in cold benzene (120 mL) was added dropwise to a stirred solution of CH_2N_2 (14.0 g, 0.33 mol) in Et_2O (500 mL) at 5–10 °C, during which time the diazo ketone separated. After the solution was stirred at 20 °C for 2 h, solvents were removed under reduced pressure below 30 °C. The residue was dissolved in dry MeOH (500 mL), and the stirred solution was heated at 55 °C and treated over 30 min with a slurry of freshly prepared Ag_2O (4.5 g) in MeOH (50 mL). The mixture was then heated under reflux for 30 min, filtered, and evaporated under reduced pressure. Crystallization of the residue from aqueous MeOH (3×) and then from benzene/light petroleum gave methyl 5-(methoxycarbonyl)-2-nitrophenylacetate (48) (13.6 g, 48%), mp 69 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 8.21 (d, J = 8.5 Hz, 1 H, H-3), 8.17 (d, J = 1.6 Hz, 1 H, H-6), 8.10 (dd, J = 8.5, 1.9 Hz, 1 H, H-4), 4.18 (s, 2 H, CH_2), 3.92 (s, 3 H, $PhCO_2CH_3$), 3.62 (s, 3 H, CH_2COOCH_3). Anal. ($C_{11}H_{11}NO_6$) C,H,N.

A stirred mixture of 48 (11.1 g, 44 mmol), freshly distilled MeI (14.34 g, 101 mmol), and 18-crown-6 (2.91 g, 11 mmol) in dry DMF (55 mL) was treated portionwise with NaH (4.04 g, 101 mmol, 60% dispersion in mineral oil) over 15 min below 5 °C and then stirred for a further 1 h at 5 °C and 6 h at 20 °C. The mixture was then diluted with CH_2Cl_2 (250 mL), and the organic layer was washed successively with dilute HCl, dilute aqueous $KHCO_3$, and water (3×) and evaporated. The residue was chromatographed on silica gel. Elution with light petroleum removed mineral oil and impurities, and elution with light petroleum/CH_2Cl_2 (3:7) gave methyl 2-[5-(methoxycarbonyl)-2-nitrophenyl]-2-methylpropanoate (49) (7.81 g, 63%), mp (light petroleum) 99–100 °C: ^{1}H NMR ($CDCl_3$) δ 8.28 (d, J = 1.7 Hz, 1 H, H-6), 8.06 (dd, J = 8.4, 1.7 Hz, 1 H, H-4), 7.93 (d, J = 8.4 Hz, 1 H, H-3), 3.98 (s, 3 H, $ArCOOCH_3$), 3.66 (s, 3 H, $C(CH_3)_2COOCH_3$), 1.71 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{13}H_{15}NO_6$) C,H,N.

The above diester (49) (7.87 g, 28 mmol) was suspended in a mixture of 2 N aqueous NaOH (84 mL) and MeOH (84 mL), and the mixture was stirred and heated under reflux until homogeneous. The MeOH was then distilled out, and the mixture was heated under reflux for a further 1 h and then cooled and acidified to give 2-(5-carboxy-2-nitrophenyl)-2-methylpropanoic acid (50) (6.68 g, 94%), mp (aqueous MeOH) 254–256 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 13.1 (v br s, 1 H, COOH), 8.21 (d, J = 1.3 Hz, 1 H, H-6), 7.99–8.06 (m, 2 H, H-3, H-4), 1.62 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{11}H_{11}NO_6$) C,H,N.

A solution of the acid (50) (6.68 g, 26 mmol) in MeOH (40 mL) and concentrated H_2SO_4 (4 mL) was kept at 20 °C for 24 h and then stirred into ice/water to give 2-[5-(methoxycarbonyl)-2-nitrophenyl]-2-methylpropanoic acid (51) (6.56 g, 93%), mp (aqueous MeOH) 162–163 °C: ^{1}H NMR ($CDCl_3$) δ 8.29 (d, J = 1.8 Hz, 1 H, H-6), 8.07 (dd, J = 8.4, 1.8 Hz, 1 H, H-4), 7.98 (d, J = 8.4 Hz, 1 H, H-3), 6.4 (br s, 1 H, CO_2H), 3.98 (s, 3 H, CH_3), 1.74 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{12}H_{13}NO_6$) C,H,N.

Reaction of 51 with 4-methoxyaniline using method B above (but replacing Na_2CO_3 with $KHCO_3$) gave N-(4-methoxyphenyl)-2-[5-(methoxycarbonyl)-2-nitrophenyl]-2-methylpropanamide (52) (88% yield), mp (aqueous MeOH) 185–186 °C: ^{1}H NMR ($CDCl_3$) δ 8.35 (d, J = 1.6 Hz, 1 H, H-6), 8.08 (dd, J = 8.3, 1.7 Hz, 1 H, H-4), 7.86 (d, J = 8.3 Hz, 1 H, H-3), 7.32 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.06 (br s, 1 H, NH), 6.85 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.99 (s, 3 H, CO_2CH_3), 3.78 (s, 3 H, OCH_3), 1.81 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{19}H_{20}N_2O_6$) C,H,N.

The ester (52) was hydrolyzed in hot NaOH/MeOH/H_2O to give N-(4-methoxyphenyl)-2-(5-carboxy-2-nitrophenyl)-2-methylpropanamide (53) (91% yield), mp (aqueous MeOH) 216–219 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 13.6 (v br s, 1 H, CO_2H), 9.13 (s, 1 H, NH), 8.25 (d, J = 1.5 Hz, 1 H, H-6), 8.03 (dd, J = 8.3, 1.5 Hz, 1 H, H-4), 7.93 (d, J = 8.3 Hz, 1 H, H-3), 7.34 (d, J = 9.0 Hz, 2 H, H-2′,6′), 6.86 (d, J = 9.0 Hz, 2 H, H-3′,5′), 3.71 (s, 3 H, OCH_3), 1.70 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{18}H_{18}N_2O_6$) C,H,N.

A stirred solution of 53 (394 mg, 1.1 mmol) in DMF (0.6 mL) was treated with 1,1′-carbonyldiimidazole (196 mg, 1.2 mmol) at 20 °C for 30 min and then cooled to 0 °C and treated with N,N-dimethylethylenediamine (125 mg, 1.4 mmol). The mixture was allowed to warm to 20 °C and then diluted with aqueous Na_2CO_3. The resulting precipitate was crystallized twice from aqueous MeOH to give N-(4-methoxyphenyl)-2-[5-[[2-(dimethylamino)-ethyl]carbamoyl]-2-nitrophenyl]-2-methylpropanamide (25a) (360 mg, 77%), mp 202–203 °C: ^{1}H NMR ($(CD_3)_2SO$) δ 9.12 (s, 1 H, CONHPh), 8.74 (t, J = 5.6 Hz, 1 H, $OCNHCH_2$), 8.14 (d, J = 0.7 Hz, 1 H, H-6), 7.88–7.96 (m, 2 H, H-3,4), 7.34 (d, J = 9.1 Hz, 2 H, H-2′,6′), 6.86 (d, J = 9.1 Hz, 2 H, H-3′,5′), 3.72 (s, 3 H, OCH_3), 3.40 (q, J = 6.5 Hz, 2 H, $CH_2CH_2N(CH_3)_2$), 2.42 (t, J = 6.8 Hz, 2 H, $CH_2CH_2N(CH_3)_2$), 2.19 (s, 6 H, $N(CH_3)_2$), 1.71 (s, 6 H, $C(CH_3)_2$). Anal. ($C_{22}H_{28}N_4O_5$) C,H,N.

Reductions. Solutions (ca. 1 mg/mL of solvent) of the nitro compounds 7a–31a in MeOH were hydrogenated for 30–180 min over Pd–C at 20 °C. The reduction solutions were assayed by HPLC to determine the cleanliness of the reductions. The

solutions were filtered, and the solvent was removed under reduced pressure at 20 °C or below. Stock solutions of the resulting amines **7b–31b** were prepared by dissolving a known weight in MeOH; these were stored at −18 °C. The stability of the bulk solutions were determined by HPLC, with fresh bulk solutions of the amine being prepared on detection of cyclization products in the solutions.

Kinetic Measurements. UV/Vis Spectrophotometry. Buffers having a constant ionic strength of 0.01 mol L^{-1} were prepared from commercial reagent grade chemicals, using Milli-Q water, by the method of Perrin and Dempsey.[35] Measurements of pH were made using an Orion SA520 pH meter. Aliquots of buffer (3.5 mL) were thermostated in the cell compartment in stoppered cuvettes. Subsequent addition of the stock solution (5–10 μL, to give a cuvette concentration of 50 μmol L^{-1}) to the cuvette initiated the reaction, which was monitored spectrophotometrically using a Hewlett-Packard 8452A diode array spectrophotometer. The UV/Vis Kinetics Software (HP89512) supplied with the spectrophotometer enabled direct calculation of the observed pseudo-first-order rate constant, with reactions being internally blanked.

HPLC. HPLC analyses of the reaction solutions were carried out concurrently with spectrophotometric determination of the reaction rate. The HPLC assays were carried out on an Ecnosphere C-18 5-μm column with a mobile phase comprising of a mixture of MeOH and phosphate buffer (10×10^{-3} mol L^{-1}, pH 7) in ratios varying from 78% to 60% MeOH, depending upon the amino/amide under investigation. A representative HPLC time course (for cyclization of compound **16b**) is given (Figure 2). Loss of the amino/amide and appearance of the lactam and the released amine were monitored by UV at 254 nm through the use of a Hewlett-Packard HPLC, with the observed pseudo-first-order rate constant being evaluated from the loss of peak area of the amino/amide (see Figure 3 for a representative plot).

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